The 42K protein of rice dwarf virus is a post-translational cleavage product of the 46K outer capsid protein

Brief Report

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Summary. The outer capsid protein (P8) heterogeneity of rice dwarf virus (RDV) exists not only in purified virus particles, but also in RDV-infected rice, transgenic rice expressing P8, E. coli expression of P8 product and the in vitro translation products of S8. N-terminal amino acid sequencing revealed that P8 is a cleavage product of P8′. The cleavage occurs specifically at the residues of Asp³⁶² and Pro³⁶³. The function of the proteolytic processing is unknown.

The 42K protein of rice dwarf virus (RDV) is a member of the phytoreovirus genus of the family Reoviridae [2]. It has a genome composed of 12 dsRNA segments designated S1 to S12 according to their mobility in polyacrylamide gel electrophoresis (PAGE). Each segment contains one single open reading frame (ORF) except S12 which has three overlapping ORFs [21]. The nucleotide sequences full genome of the Japanese isolate and Chinese isolate of RDV have been determined [24–26].

The intact particles of RDV contain seven structural proteins P1, P2, P3, P5, P7, P8 and P8′ with respective sizes of 170, 130, 110, 89, 58, 46, and 42K. These proteins have been confirmed as the products of S1, S2, S3, S5, S7, and S8 [22] and the localizations of these proteins are core, outer capsid, major core, minor core, outer capsid and outer capsid, respectively [15, 16]. Suzuki and Sugawara [20] investigated the 46K (P8) and 42K (P8′) outer capsid proteins, using peptide mapping and electroblot-ELISA with purified RDV and two specific monoclonal antibodies against the 46K and 42K proteins. They concluded that the two proteins
have similar structures and epitopes and are overlapping products. Since segment S8 of RDV contains one single open reading frame (ORF) with a size of 1236bp, Suzuki and Sugawara suggested that the 42K protein is either a post-translation cleavage product from the 46K protein or is translated from the third in-frame initiation codon (bases 147 to 149) on S8 [21]. Recently, we have detected the P8′ (42K) protein in RDV-infected rice plants and transgenic rice expressing RDV P8 [28], E. coli expressing proteins from the S8 ORF in the expression vector pBV220[27] and in vitro translation products of S8 (Figs. 1 and 4). Therefore, we decided to investigate the mechanism generating the P8′ protein.

Proteins from purified RDV preparations, P8 and P8′ expressed in E. coli were examined. RDV was purified according to the method of Gao [4]. Total proteins from E. coli were extracted according to the method of Laemmli [11]. Proteins both from purified virus particles and E. coli expression product were extracted and denatured by 5 min incubation at 100 °C in 50 mM Tris-HCl pH 6.8 containing 2% SDS, 5% 2-mercaptoethanol and separated in 2%-SDS-10%-polyacrylamide gel electrophoresis [7, 11, 12]. After electrophoresis the proteins were electroblotted onto polyvinylidenefluoride (PVDF) membrane [5], then the appropriate bands were excised and loaded into Procise ABI amino acid sequencer. The N-terminal amino acid sequence of 42K bands from RDV purified particles and from E. coli expression were both “MSRQMWLDFS”. This sequence matches exactly the first nine amino acids of 46K protein of P8 deduced...